European Journal of Medicinal Chemistry 46 (2011) 3030–3037

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Novel aliphatic *N*-oxide of naphthalimides as fluorescent markers for hypoxic cells in solid tumor

Hong Yin, Weiping Zhu, Yufang Xu*, Min Dai, Xuhong Qian*, Yuanli Li, Jianwen Liu

Shanghai Key Laboratory of Chemical Biology, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, PR China

ARTICLE INFO

Article history:
Received 31 July 2009
Received in revised form
13 April 2011
Accepted 13 April 2011
Available online 21 April 2011

Keywords:
Naphthalimide
Fluorescent marker
Hypoxic
N–Oxide
Bioreduction

ABSTRACT

A series of novel aliphatic *N*-oxide of naphthalimides (**A1**–**A5**) were designed and prepared. The N–O group was firstly introduced into the amine side chain tailed to planar naphthalimide chromophore as hypoxic bioreductive marker. Fluorescence image analysis showed that the compounds could be used as potential markers for hypoxic cells (V79) in vitro especially for **A1** with 17 times hypoxic-oxic fluorescence differential, which was probably due to the bis-bioreduction mechanism.

© 2011 Elsevier Masson SAS. All rights reserved.

1. Introduction

The hypoxia arose in many tumors because of inefficient delivery of oxygen via the poorly organized tumor vasculature [1,2,3]. It was clear that tumor hypoxia reduced the efficacy of radiotherapy and in some cases contributed to local failure of radiotherapy and tumor regrowth. Hypoxia was also involved in many common types of normal tissue morbidity [4]. Therefore, a simple method for measuring tumor hypoxia could diagnose tumors and facilitate optimal treatment schedules for individual patients, with the use of modalities designed to overcome or take advantage of the presence of hypoxic cells in their tumor [5].

A number of methods were proposed for measuring hypoxic cell fraction in tumors, such as oxygen microelectrodes, histomorphometric analysis and DNA strand breaks. However, most of them were invasive and not readily available to most investigators [6]. Over the past two decades, one simpler, easier and non-invasive method for hypoxic cells detection was developed through fluorescent agents, such as 2-nitroimidazole hypoxic markers and nitroaromatic compounds [7]. Some successful examples were shown in Fig. 1.

The nitro-compounds (Fig. 1) were suggested for identifying hypoxic cells by fluorescence [8,9,10,11,12,13,14]. The nitro group quenched the fluorescence of the aromatic ring system through energy transfer and electron transfer. But the compound became more fluorescent on bioreduction of the nitro group to amino group [12]. Thus the hypoxia-specificity response was resulted from both the hypoxia-dependence of nitro-group bioreduction and the increase in fluorescence from bioreduction. Usually, the hypoxic markers used in model experiments in vitro had large planar aromatic structures and good intercalating properties [11,12,15], their fluorescent metabolites localized into the nuclear region of cells because of their affinity to DNA. The naphthalimide derivatives were reported as antitumor agents [16] with the strong ability of binding with DNA, which were first discovered by Braña and co-workers, and the two famous compounds known as amonafide and mitonafide had been selected for Phase II clinical trials. Hence, in this paper, we selected naphthalimide as the DNA binding part for designing novel markers in solid tumors.

In hypoxic cells, the tertiary amine *N*-oxides could release the active cytotoxins primarily by the CYP3A isozyme of NADPH: cytochrome C (P-450) reductase was inhibited by oxygen, probably because of direct competition between oxygen and the drug at the enzyme site [17]. After the *N*-oxides being bioreduced to the corresponding tertiary aliphatic amines, they could bind with DNA and interfere with topoisomerase function [18,19,20]. But the most interesting thing is that the fluorescence of the *N*-oxides could

* Corresponding authors. Tel.: +86 21 64253589; fax: +86 21 64252603.
E-mail addresses: yfxu@ecust.edu.cn (Y. Xu), xhqian@ecust.edu.cn (X. Qian).

0223-5234/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2011.04.040

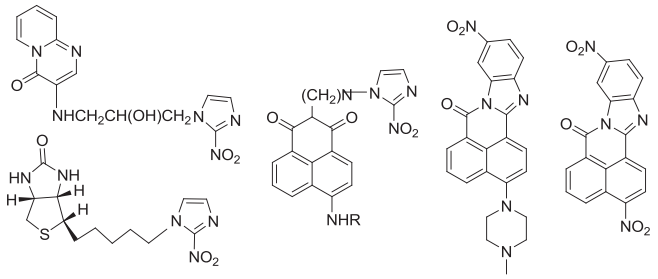


Fig. 1. Structures of some reported markers for hypoxic cells in solid tumors.

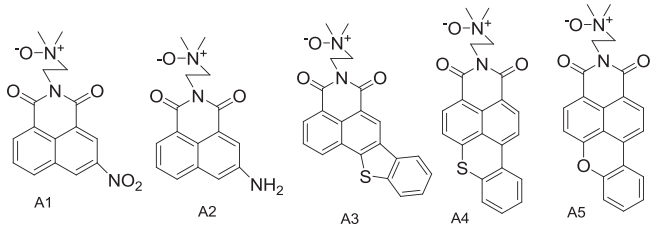
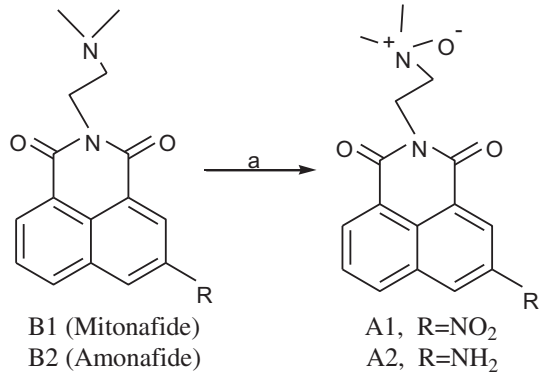
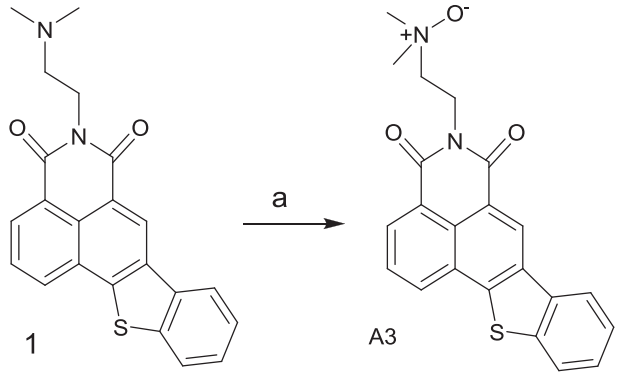


Fig. 2. Novel compounds for potential hypoxic fluorescent markers (**A1**–**A5**).



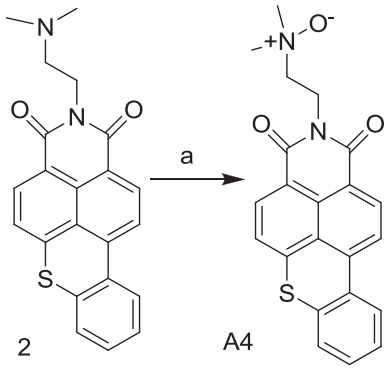
Scheme 1. (a) H_2O_2 (30%), CH_2Cl_2, reflux, 1 h.



Scheme 2. (a) H_2O_2 (30%), CH_3OH, reflux, 1–3 h.

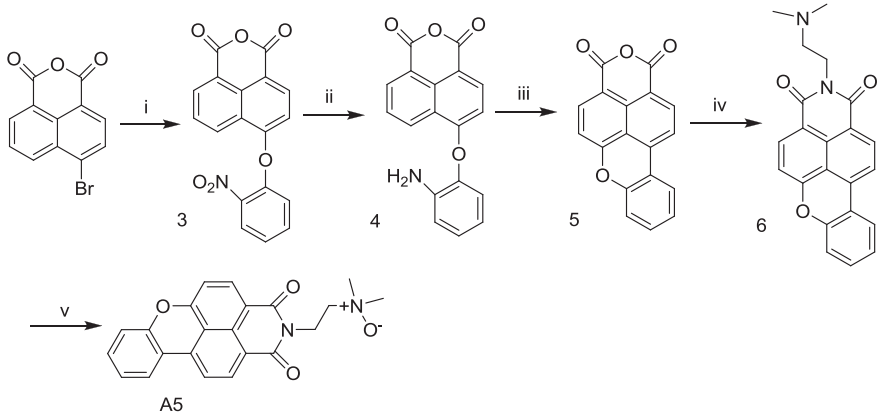
change much after bioreduction (generally for PET or ICT mechanism), and the reduced amines could ensure good uptake into cells and tight binding with DNA.

With these in mind, we presented a series of novel markers for hypoxic cells in solid tumors from tertiary amine *N*-oxide of



Scheme 3. (a) H_2O_2 (30%), CH_3OH, reflux, 5 h.

naphthalimides (Fig. 2), such as amonafide, mitonafide and other antitumor agents. Herein, N–O group was firstly introduced to the amine side chain tailed to planar naphthalimide chromophore to identify hypoxic cells. For compound **A1**, with two active sites for bioreduction, we expect that it should show higher selectivity than **A2** and other compounds. Meanwhile, the properties of these compounds as fluorescent markers were also evaluated.



Scheme 4. (i) *o*-nitrophenol, NaOH, DMF, Cu, reflux 1 h; (ii) Fe, acetic acid, reflux 1 h; (iii) hydrochloric acid, acetic acid, $NaNO_2$, 0–5 °C, $CuSO_4$, HOAc, H_2O; (iv) *N*,*N*-dimethylethylenediamine, ethanol, reflux 2–3 h; (v) H_2O_2 30%, CH_3OH, reflux, 5 h. 0.64 g (86%).

Table 1
Spectral data of **A1**–**A5** and corresponding amino compounds.[a,b]

Compounds	UV λ_{max} (lgϵ)	FL λ_{max} (Φ)
A1	331 (4.04)	368 (<0.001)
A2	343 (3.53)	548 (0.94)
A3	379 (4.14)	461 (0.90)
A4	461 (4.40)	522 (0.17)
A5	425 (4.38)	473 (0.143)
B1	316 (3.93)	368 (<0.001)
B2	342 (3.98)	547 (0.03)
1	378 (4.0)	456 (0.26)
2	460 (4.34)	510 (0.09)
6	424 (4.18)	472 (0.03)

[a] In absolute ethanol.
[b] With fluorescence in sodium hydrate solution as quantum yield standard ($\lambda_{ex/nm}$ = 366 nm, φ = 0.97).

2. Results and discussion

2.1. Chemistry

The target compounds were synthesized by using 1,8-naphthalic anhydride and 4-bromo-1,8-naphthalic anhydride as starting material. The synthetic routes were shown in Scheme 1–4, respectively.

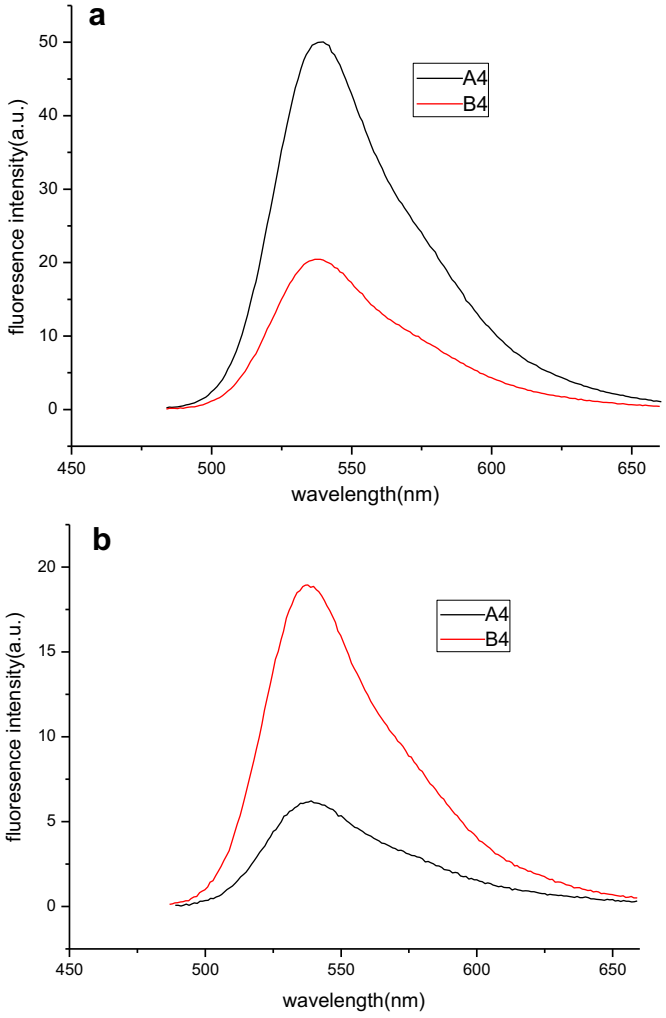


Fig. 3. Fluorescence spectra in different solvents. The concentration of compound **A4** was 10^{-4} mol/L: (a) in absolute ethanol; (b) in water (20 mM Tris–HCl, pH 7.5). The black represented A4, and the red represented the corresponding amine 2 (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

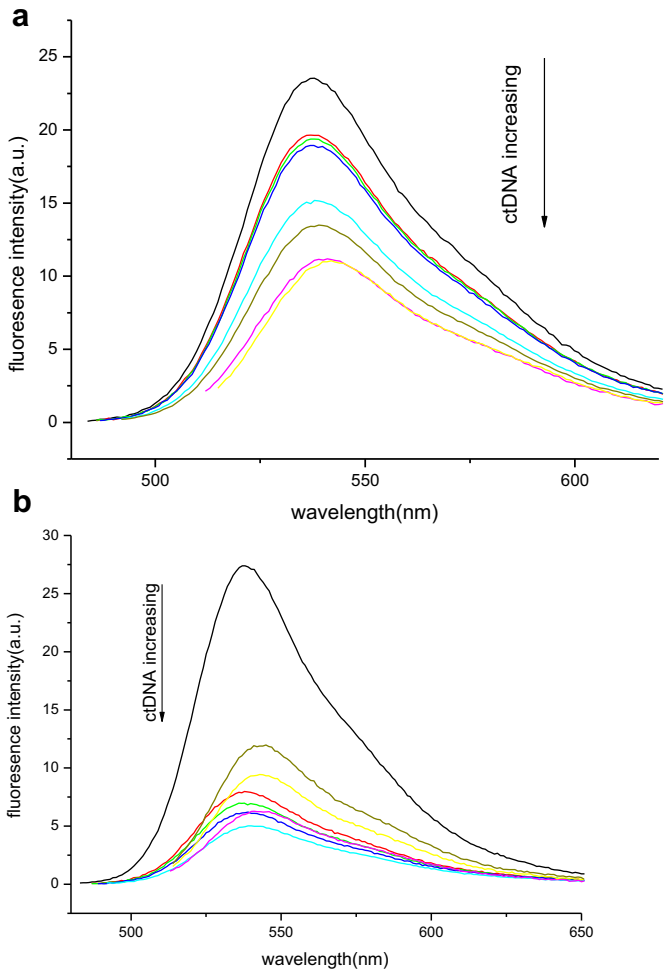


Fig. 4. Fluorescence spectra of compound **A4** (a) and the corresponding amine 2 (10×10^{-6} M); (b) with addition of ctDNA in increasing concentration ($0–400 \times 10^{-6}$ M) in 20 mM Tris–HCl (pH 7.5) at room temperature, the excitation wavelength was 460 nm.

The following listed the synthetic steps for compound **A5** as an example: 4-bromo-1,8-naphthalic anhydride and *o*-nitrophenol were dissolved in DMF and stirred for 1 h under reflux with NaOH and Cu as catalysts to give a yellow solid **3**. The solid **3** was treated with Fe powder in glacial acetic acid and refluxed for 1 h to afford khaki solid **4**. Then **4** was added into the hydrochloric acid and sodium nitrite at 0 ~ 5 °C for 1 h, followed by the addition of $CuSO_4$ solution and refluxed for 0.5 h to give a yellow solid benzo(k,l)xanthene-3,4-dicarboxylic anhydride **5**, which was mixed with *N*,*N*-dimethylethylenediamine in ethanol and refluxed for 2–3 h to give the important intermediate product **6**. Finally, the NO group was introduced by oxidation with H_2O_2 (30%) in CH_2Cl_2 or methanol under reflux for 1 ~ 5 h, removal of the solvent gave the desired compounds **A5** in high yields. All of the structures were confirmed by IR, ^1H NMR and HRMS. A strong N–O stretching vibration appeared in the range of 2349–2338 cm^{-1} in their infrared spectra, which was the characteristic absorption band of the *N*-oxide.

The UV–Vis and fluorescent data for these compounds were measured and shown in Table 1. It was found that the emission wavelength of the *N*-oxides was red-shifted about 4 ~ 5 nm respectively than the corresponding naphthalimides in absolute ethanol. It was probably due to the increase of electronic pushing-pulling effects caused by the introduction of the weak electron-withdrawing NO group to the side chains.

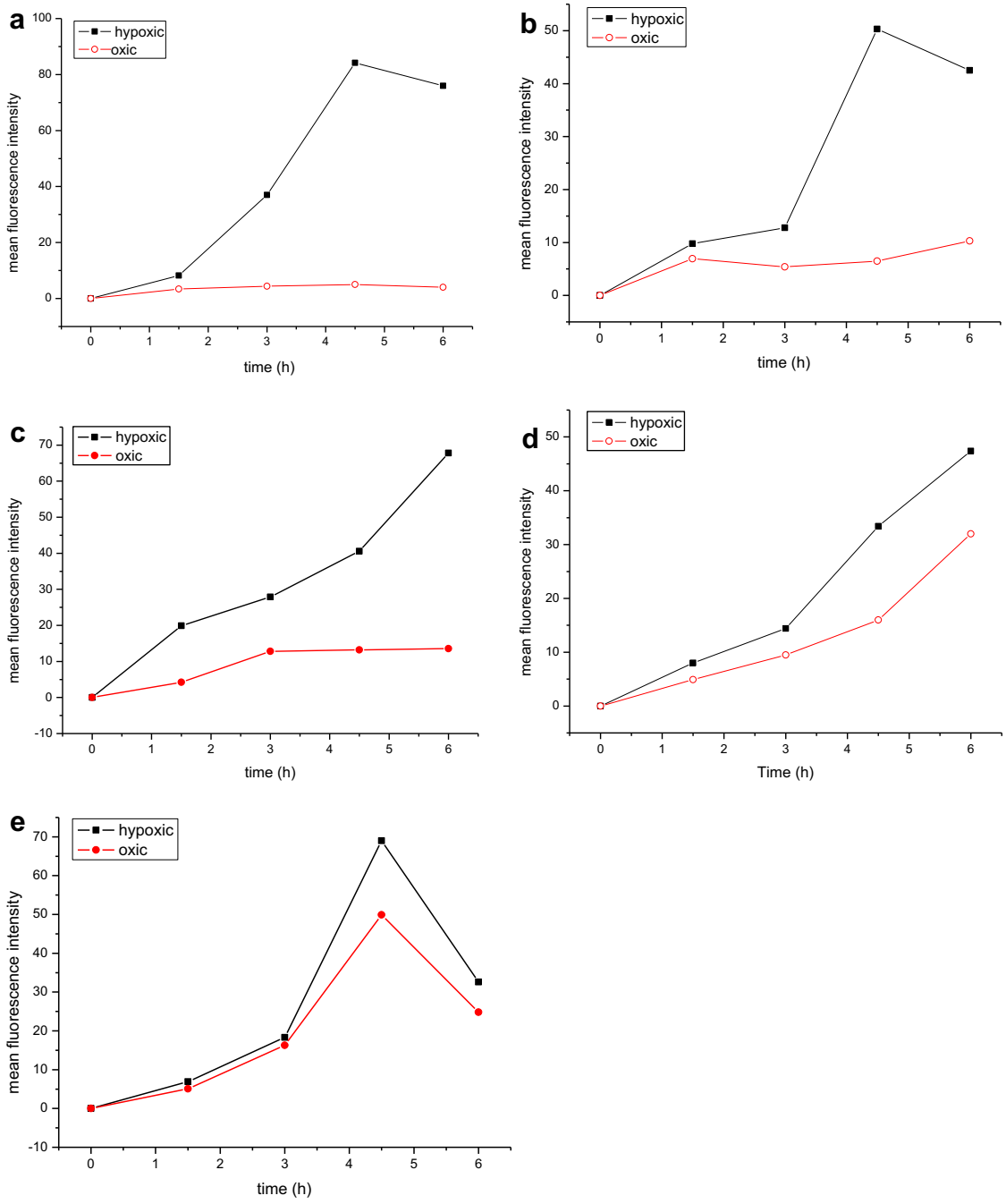


Fig. 5. The time courses of accumulation of fluorescent metabolites in V79 Chinese hamster cells incubated with 10^{-4} M compounds at 37 °C; (a) **A1** (b) **A2**(c) **A3** (d) **A4** (e) **A5**.

Meanwhile, as shown in Table 1, the quantum yields of the *N*-oxides were ranged from 0.143 to 0.94 (Φ), and that of the corresponding amines varied from 0.03 to 0.26 (Φ) except **A1**, which indicated that the fluorescent intensities changed much between the *N*-oxides and their corresponding amines. In detail, the quantum yield of **A2** was about 30 folds stronger than its former compound **B2** (amonafide), and **A3**, **A4** and **A5** were about 3.5, 1.9, 8.2 folds stronger than their corresponding amines respectively. The reason was probably that the former amines with nitrogen in the tertiary amine side chains generated Photo-induced Electron Transfer (PET) between the planar aromatic ring and the side chain. When the nitrogen atom on the side chain was oxidized, the property of the group changed from electron donating to electron withdrawing, which probably inhibited PET effect to some extent, and it resulted in a high quantum yield than the corresponding amines. It provided the possibility for our design strategy as fluorescence markers.

In addition, the fluorescence of the target compounds (representative curves for **A4**) in ethanol and water was evaluated respectively as shown in Fig. 3.

In Fig. 3(a), it was found that the fluorescence intensity of **A4** in absolute ethanol was stronger than that of the related amino compound **2**, but it was converse in water (Fig. 3b). The reasons were probably associated with several aspects as follows: firstly, the PET effect of the amino compound **2** was stronger than **A4** to quench the fluorescence in ethanol because of the weak protonic ability; Secondly, the aliphatic *N*-oxide **A4** with more charges was easier to accumulate or aggregate than its corresponding amine **2** in

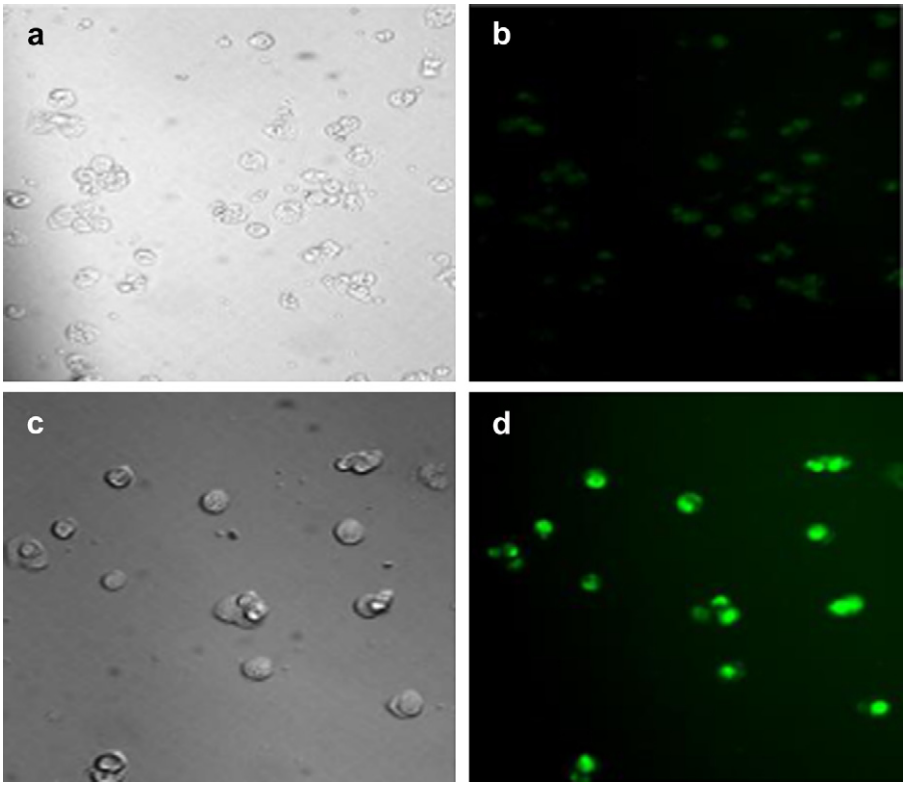


Fig. 6. Fluorescence microphotographs of V79 cells incubated with 10^{-4} M of **A1** at 37 °C. After 4.5 h incubation, scanning was taken. Magnification was 1000×. (a) scanning was taken on brightfield cells on oxic condition (incubated in air and 5% CO_2); (b) excited at 410 nm, cells on oxic condition; (c) scanning was taken on brightfield, cells on hypoxic condition (incubated in nitrogen and 5% CO_2); (d) excited at 410 nm, cells on hypoxic condition.

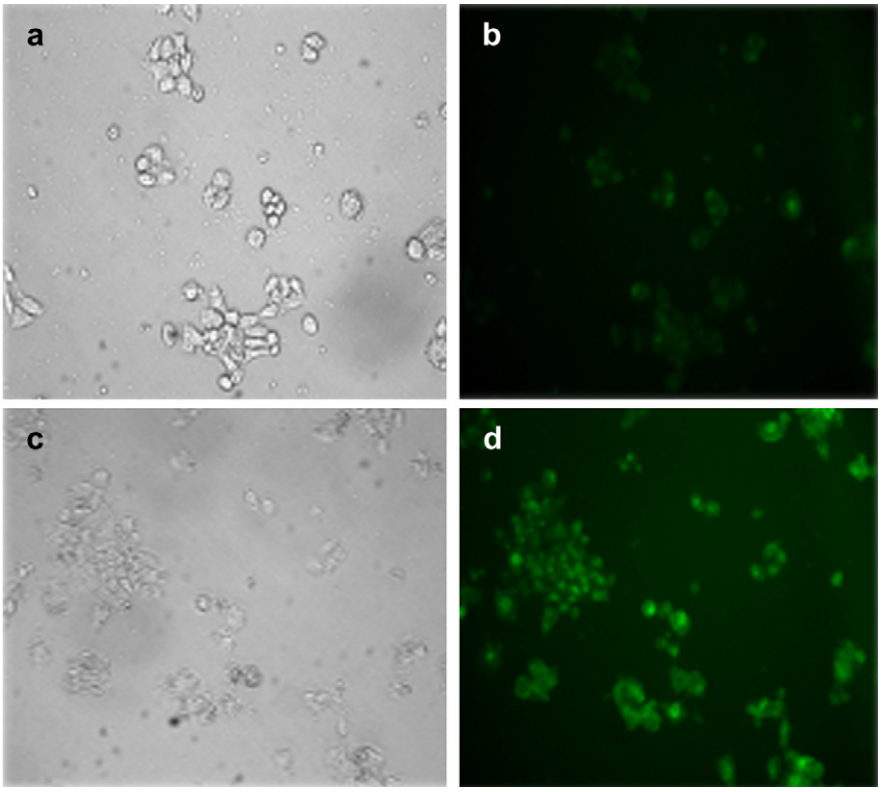


Fig. 7. Fluorescence microphotographs of V79 cells incubated with 10^{-4} M of **A2** at 37 °C. After 4.5 h incubation, scanning was taken. Magnification was 1000×. (a) scanning was taken on brightfield cells on oxic condition (incubated in air and 5% CO_2); (b) excited at 410 nm, cells on oxic condition; (c) scanning was taken on brightfield, cells on hypoxic condition (incubated in nitrogen and 5% CO_2); (d) excited at 410 nm, cells on hypoxic condition.

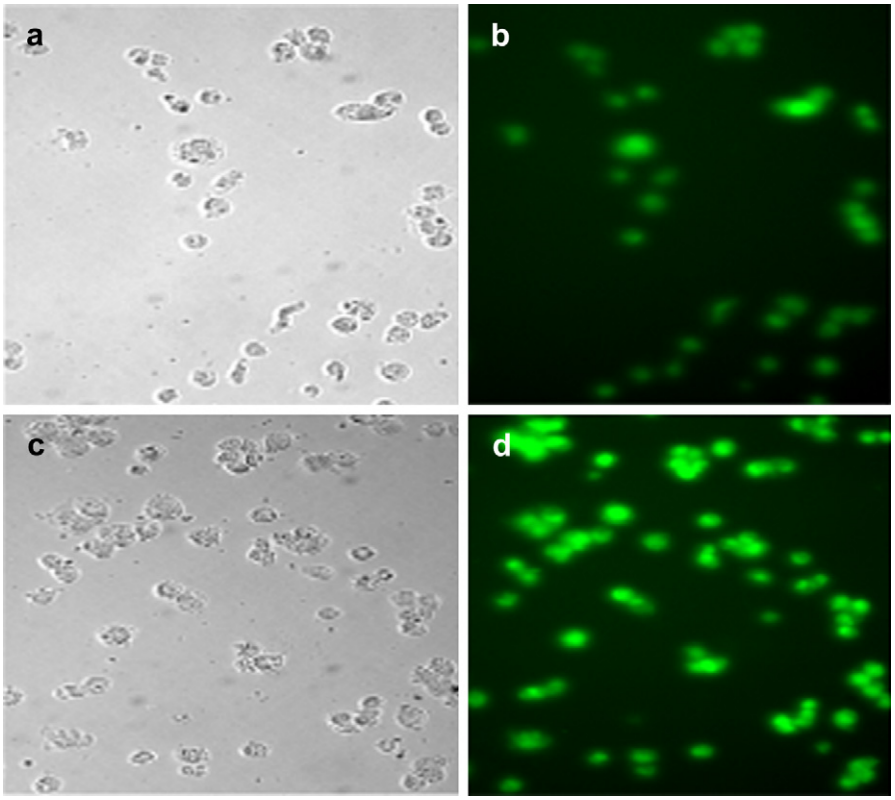


Fig. 8. Fluorescence microphotographs of V79 cells incubated with 10^{-4} M of **A4** at 37 °C. After 4.5 h incubation, scanning was taken. Magnification was 1000×. (a) scanning was taken on brightfield cells on oxic condition (incubated in air and 5% CO_2); (b) excited at 410 nm, cells on oxic condition; (c) scanning was taken on brightfield, cells on hypoxic condition (incubated in nitrogen and 5% CO_2); (d) excited at 410 nm, cells on hypoxic condition.

water, which resulted in the fluorescence quenched much of compound **A4** [21]; Thirdly, different hydrogen bonding with water would affect their fluorescence intensity: the stronger of the ability, the weaker of the fluorescence.

2.2. DNA binding property

The binding properties between the compounds and ctDNA were evaluated (Fig. 4). The fluorescence data were analyzed to give the binding constant of **A4** and its corresponding amine **2** about 6.62×10^3 M^{-1} and 2.9×10^5 M^{-1} respectively [22]. It was about 15 folds lower of the *N*-oxide than its former, which might be caused by the disappearance of hydrogen bond between nitrogen and DNA. It implied that weaker ctDNA binding probably endowed the *N*-oxides lower toxicity and extravascular drug transport properties [20].

2.3. Evaluation as fluorescence marker

For the evaluation of these compounds as fluorescence markers, V79 cell which was more sensitive to fluorescence [23] was selected. Samples from hypoxic and oxic cell suspensions incubated with the solution of compounds were taken, and the study of the time courses of accumulation of fluorescent metabolites in V79 cells incubated with 10^{-4} M of **A1**–**A5** at 37 °C was carried out with fluorescence scan ascent. The results were shown in Fig. 5.

In Fig. 5, it was found that obvious fluorescence differential of cells could be seen under hypoxic and oxic conditions. After about 4.5 h, the hypoxic-oxic fluorescence differential reached the maximal value. The hypoxic-oxic ratio of **A1**–**A5** in the fluorescence images was about 17, 8.4, 5.2, 1.75, 1.5 times respectively. The largest hypoxic-oxic ratio of **A1** in V79 cells (17 times) was higher than that of other compounds. The reason was probably due to that **A1** was a "bis-bioreductive" [24] with two different independent oxygen-sensitive redox centers: the nitro group and the NO group. Under the oxygen-inhabitable reduction, the NO group was reduced by CYP3A isozyme of NADPH: cytochrome C (P-450) reductase. In hypoxic cells, the fluorescence quenching group (NO_2) was reduced to amine by nitroreductase enzymes in 2-electron steps [25], and the chromophore changed from pulling–pulling system to pulling-pushing one, which resulted in fluorescence enhancement markedly. Therefore, it could be deduced that the bioreduction of the nitro-group contributed to the higher fluorescence differential of **A1**. Compared with **A1**, the bioreduction of the N–O group resulted in less hypoxic-oxic fluorescence differential for **A2** and **A4**. The hypoxic-oxic fluorescence differential incubated with **A3** and **A5** was evaluated as 5.2 and 1.5 times, respectively.

By fluorescence microscopy, we also got the fluorescence microphotographs of V79 cells incubated with 10^{-4} M of **A1**, **A2** and **A4**, respectively (Figs. 6–8). Because the fluorescence intensities of the bioreductive products of compounds **A4** and **A5** are not strong, the high concentrations of the fluorescent probes (10^{-4} M) were used in the experiments. Figs. 6–8 showed that the microphotographs of **A1** showed higher differential fluorescence between hypoxic and oxic V79 cells than other *N*-oxides did, which was in accordance with the differential evaluated with fluorescence scan ascent above. It was also found that the fluorescence intensities in hypoxic cell cultures were stronger than that in oxic cells. The results showed that the metabolized products (naphthalimides) in hypoxic culture medium exhibited stronger fluorescence intensities than that in oxic cell cultures, where the *N*-oxides could not be metabolized, which was in agreement with the fluorescence change in water (Fig. 3).

3. Conclusion

In summary, we described the synthesis and DNA-binding affinities of the novel aliphatic *N*-oxide of naphthalimides, and investigated their abilities as fluorescent markers for hypoxic cells in solid tumors. From the fluorescence images, the target compounds were shown to be good markers for hypoxia cells (V79) in vitro especially for compound **A1**, which was probably due to bis-bioreduction with 17 times hypoxic-oxic fluorescence differential. The other *N*-oxides with differential of 8.4, 5.2, 1.75 and 1.5 times were also promising candidate markers for hypoxic cells.

4. Experimental protocols

All the solvents were of analytic grade. ^{1}H NMR was measured on a Bruker AV-500 spectrometer with chemical shifts reported as parts per million (in acetone-d_6/DMSO-d_6/$CDCl_3$, TMS as an internal standard). Mass spectra were measured on an HP 1100 LC-MS spectrometer. Melting points were determined by an X-6 micro-melting point apparatus and uncorrected. Absorption spectra were determined on PGENERAL TU-1901 UV–VIS Spectrophotometer.

4.1. Synthesis of naphthalimide derivatives

4.1.1. N-Oxide 3-nitro-1,8-naphthalimide (**A1**)

H_2O_2 (30%, 3 mL, 30 mmol) was added drop-wise to the 3–NO_2-naphthalimide (0.94 g, 3 mmol) in methylene chloride (30 mL), and the mixture was refluxing for 3 h. After removing most of the solvent, ethyl acetate was added. The yellow solid was precipitated, filtered and separated on silica gel ($CHCl_3$/methanol = 10:2), which yielded pale yellow solid 0.79 g (80%). m.p.184–185 °C .^{1}H NMR (CD_3OD, ppm) δ_H: 3.33 (s, 6H, $NOCH_3$), 3.70 (t, J = 7.00 Hz, 2H, $NOCH_2$), 4.69 (t, J = 6.85 Hz, 2H, $CONCH_2$), 8.00 (t, J = 7.65 Hz, 1H, 2-H), 8.63 (d, J = 8.26 Hz, 1H, 1-H), 8.77 (d, J = 7.24 Hz, 1H, 3-H), 9.19 (d, J = 2.14 Hz, 1H, 9-H), 9.33 (d, J = 2.10 Hz, 1H, 7-H); IR (KBr, cm^{-1}): 3069, 2353, 2338, 1664, 1341, 800; HRMS: $C_{21}H_{16}O_5N_3$ $(M + H)^+$ calculated 330.1130, found 330.1103.

4.1.2. N-Oxide 3-amino-1,8-naphthalimide (**A2**)

3–NH_2-naphthalimide (0.85 g, 26 mmol) was dissolved in methylene chloride (25 mL), and H_2O_2 (30%, 2.7 mL, 26 mmol) was added dropwise. The mixture was stirred and refluxed for 3 h. After the solvent was removed, the ethyl acetate was added to give the orange solid. The solid was filtered and separated on silica gel ($CHCl_3$/methanol = 10:1), which gave the pure product 0.76 g (85%). m.p. 160–161 °C ^{1}H NMR (CD_3OD, ppm) δ_H: 3.30 (s, 6H, $NOCH_3$), 3.64 (t, J = 7.13 Hz, 2H, $NOCH_2$), 4.63 (t, J = 6.96 Hz, 2H, $CONCH_2$), 7.35 (d, J = 2.38 Hz, 1H, 9-H), 7.57 ~ 7.61 (m, 1H, 1-H), 7.98 (t, J = 4.51 Hz, 1H, 2-H), 8.03 ~ 8.04 (m, 1H, 7-H), 8.18 ~ 8.20 (m, 1H, 3–H); IR (KBr, cm^{-1}): 3416, 3338, 2353, 2326, 1649, 1622, 789; HRMS: $C_{16}H_{18}O_3N_3$ $(M + H)^+$ calculated 300.1348, found 300.1333.

4.1.3. N-Oxide Benzo[b]xanthene [2,1-c]naphthalimide (**A3**)

Compound **1** was synthesized as previously reported [26]. H_2O_2 (30%, 0.2 mL, 20 mmol) was added dropwise to the solution of compound **1** (0.75 g, 2 mmol) in methanol (30 mL). The mixture was refluxed for 5 h, with viscousness on the bottle. After the solvent was removed, ethyl acetate was added. The solid was filtered and separated on silica gel ($CHCl_3$/ethanol = 10:2) to give pale yellow solid 0.7 g (90%). m.p. 152–153 °C ^{1}H NMR (CD_3OD, ppm) δ_H: 3.35 (s, 6H, $NOCH_3$), 3.68 (t, J = 7.27 Hz, 2H, $NOCH_2$), 4.58 (t, J = 7.04 Hz, 2H, $CONCH_2$), 7.50 ~ 7.54 (m, 2H, 9-H, 10-H), 7.66 (t, J = 7.74 Hz, 1H, 2-H), 7.87 ~ 7.89 (m, 1H, 8-H), 8.10 ~ 8.12 (m, 1H, 1-H), 8.21 (d, J = 7.42 Hz, 1H, 11-H) 8.35 (d, J = 7.31 Hz, 1H, 3-H) 8.76 (s, 1H, 7-H); IR (KBr, cm^{-1}): 3326, 2365, 2338, 1696, 1660, 1334, 734; HRMS: $C_{22}H_{18}O_3N_2$: $(M + H)^+$ calculated 391.1116, found 391.1119.

4.1.4. N-Oxide Benzo[k,l]thioxanthene-3,4-naphthalimide (**A4**)

Compound **2** was synthesized as previously reported [27]. H_2O_2 (30%, 0.2 mL, 20 mmol) was added dropwise to the naphthalimide **2** (0.75 g, 20 mmol) in methanol (30 mL), and the mixture was refluxed for 5 h, with viscousness on the bottle. After the solvent was removed, ethyl acetate was added. And the viscousness solid was filtered and separated on silica gel ($CHCl_3$/methanol = 10:2) to give black red solid 0.61 g (78%). m.p. 159–160 °C ^{1}H NMR (CD_3OD, ppm) δ_H: 3.32 (s, 6H, $NOCH_3$), 3.66 (t, J = 7.20 Hz, 2H, $NOCH_2$), 4.65 (t, J = 7.04 Hz, 2H, $CONCH_2$), 7.45 (s, 3H, 9-H, 10-H, 11-H), 7.58 (d, J = 8.03 Hz, 1H, 8-H) 8.34 (d, J = 8.08 Hz, 3H, 1-H, 2-H, 7-H), 8.54 (d, J = 8.20 Hz, 1H, 6-H); IR (KBr, cm^{-1}): 3350, 2361, 2338, 1688, 1649, 1583, 1369, 758.1; HRMS: $C_{22}H_{18}O_3N_2$ $(M + H)^+$ calculated 391.1116, found 391.1114.

4.1.5. 4-(2-Nitrophenoxy)-1,8-naphthalic anhydride (**3**)

A mixture of 4-bromonaphthalic anhydride (1.02 g, 4.4 mmol), *o*-nitrophenol (0.34 g, 8.5 mmol), sodium hydroxide (0.025 g) and copper powder (0.04 g) was refluxed in DMF (45 mL) for 1 h. Hydrochloric acid (7.5 mL, 20%) was added to the solution and the solid was precipitated, filtered and recrystallized in AcOH to afford the title compound in 82% yield. m.p. 268–269 °C, and m.p. 266–268 °C in literature [28].

4.1.6. 4-(2-Aminophenoxy)-1,8-naphthalic anhydride (**4**)

A mixture of compound **3** (0.25 g, 0.8 mmol) and iron powder (0.12 g, 2 mmol) was refluxed in glacial acetic acid (10 mL) for 1 h. To this brown solution, water (30 mL) was added. The precipitated yellow solid was filtered and washed with water to afford the title compound in 94% yield. m.p. 172–174 °C, and m.p.171–172 °C in literature [28].

4.1.7. Benzo[k,l]xanthene-3,4-dicarboxylic anhydride (**5**)

A solution of compound **4** (0.5 g, 1.7 mmol) in glacial acetic acid (12 mL) was treated with hydrochloric acid (1 mL) and sodium nitrite (1.14 g, 16 mmol in 4 mL water) at 0 °C. After 60 min, a solution of copper sulfate (1.12 g, 7 mmol) in water (20 mL) was added. The mixture was refluxed for another 0.5 h and then allowed to cool. The precipitated solid was filtered, washed with water and crystallized in DMF to afford the title compound, yield 93.2%. m.p. 156–159 °C, and m.p. 155–160 °C in literature [28].

4.1.8. Benzo[k,l]xanthene-3,4-naphthalimide (**6**)

Compound **5** was dissolved in 20 mL absolute ethanol, with *N, N*-dimethylethylenediamine added, the mixture was stirred and refluxed for 2–3 h. Then the solution was evaporated in vacuum, and the residue was purified on silica gel chromatography, eluting with ($CHCl_3$/ethanol, 10:1) to yield the **6**, 0.314 g (85%). m.p. 191–192 °C; ^{1}H NMR ($CDCl_3$, ppm) δ_H: 2.43–2.46 (m, 6H, $N(CH_3)_2$), 2.75 (s, 2H, $CH_2N(CH_3)_2$), 4.38 (t, J = 7.06 Hz, 2H, $CONCH_2$), 7.29 (d, J = 8.30 Hz, 1H, 11-H), 7.35 (d, J = 7.37 Hz, 1H, 8-H), 7.36 (t, J = 7.92 Hz, 1H, 9-H), 7.54 (t, J = 7.17 Hz, 1H, 10-H), 7.95 (d, J = 7.89 Hz, 1H, 7-H), 8.08 (d, J = 7.90 Hz, 1H, 1-H), 8.59 (d, J = 8.29 Hz, 1H, 6-H), 8.64 (d, J = 7.88 Hz, 1H, 2-H); IR (KBr, cm^{-1}): 2937 , 2770 , 1645 , 1594 , 1380; HRMS: $C_{22}H_{18}N_2O_3$ calculated 358.1317, found 358.1321.

4.1.9. N-Oxide Benzo[k,l]xanthene-3,4-naphthalimide (**A5**)

H_2O_2 (30%, 0.2 mL, 20 mmol) was added dropwise to **6** (0.74 g, 2 mmol) in methanol (30 mL), the mixture was refluxing for 5 h with viscousness on the bottle. After the solvent was removed, ethyl

acetate was added. The viscousness solid was filtered and separated on silica gel ($CHCl_3$/methanol = 10:1) to give pale yellow solid 0.64 g (86%). m.p. 169–170 °C; ^1H NMR (CD_3OD, ppm) δ_H: 3.35 (s, 6H, $NOCH_3$), 3.69 (t, J = 7.07Hz, 2H, $NOCH_2$), 4.63 (t, J = 6.97Hz, 2H, $CONCH_2$), 7.23 (d, J = 8.33 Hz, 1H, 7H), 7.32 (d, J = 8.25 Hz, 1H, 8-H), 7.35 (t, J = 8.19 Hz, 1H, 9-H), 7.55 (t, J = 8.49 Hz, 1H, 10-H), 7.97 (d, J = 7.95 Hz, 1H, 11-H), 8.12 (d, J = 8.02 Hz, 1H, 1-H), 8.43 ~ 8.46 (m, 2H, 2-H, 6-H); IR (KBr, cm^{-1}): 3536, 2349, 2338, 1645, 1590, 1380, 777; HRMS: $C_{22}H_{18}O_4N_2$ $(M + H)^+$ calculated 391.1345, found 375.1347.

4.2. Cell culture

Compounds were initially dissolved at 1×10^{-2} M in dimethyl sulfoxide (DMSO), and small volumes were added to cell suspensions to give the appropriate concentration. The final concentration of DMSO was 1% or less.

V79 379A Chinese hamster cells were maintained as exponentially-growing suspension cultures in Eagle's Minimal Essential Medium with Earle's salts, modified for suspension cultures with 7.5% fetal calf serum. The compounds were added to cell suspensions to give the appropriate drug concentration. Then the suspension was incubated in special gases (air + 5% CO_2, nitrogen + 5%CO_2) at 37 °C. After various periods of incubation with these compounds, cell samples were removed centrifuged and washed with PBS to remove residual compound, re-suspended in a small volume of PBS, and evaluated by Fluorescence Microscopy and Fluorescence Microplate Reader using appropriate excitation wavelength.

Acknowledgements

This work was financially supported by the Key New Drug Creation and Manufacturing Program (2009ZX09103-102), the China 111 Project (grant B07023) and the Shanghai Leading Academic Discipline Project (B507) and the Shanghai Foundation of Science and Technology (073919109).

References

[1] R.S. Bush, R.D.T. Jenkin, W.E.C. Allt, F.A. Beale, H. Bean, A.J. Dembo, J.F. Prinde, Br. J. Cancer. 37 (1978) 302–306.
[2] I. Churchill-Davidson, C.A. Foster, G. Wiernik, C.D. Collins, N.C. Piezez, D.B.L. Skeggs, P.R. Purser, Br. J. Radiol. 39 (1966) 321–331.
[3] L.H. Gray, A.D. Conger, M. Ebert, S. Hornsey, O.C.A. Scott, Br. J. Radiol. 26 (1953) 638–648.
[4] R.J. Hodgkiss, Anti-Cancer Drug Des 13 (1998) 687–702.
[5] (a) J.A. Raleigh, M.W. Dewhirst, D.E. Thrall, Semin. Radiat. Oncol. 6 (1996) 37–45;
(b) M. Bache1, M. Kappler, H.M. Said, A. Staab, D. Vordermark, Curr. Med. Chem. 15 (2008) 322–338;
(c) K. Gulliksrud, I.K. Vestvik, K. Galappathi, B. Mathiesen, E.K. Rofstad, Radiat. Res. 170 (2008) 638–650;
(d) A. Cheguillaume, J. Gillart, D. Labar, V. Grégoire, J. Marchand-Brynaert, Bioorg. Med. Chem. 13 (2005) 1357–1367.
[6] (a) A.W. Fyles, M. Miolsevic, R. Wong, M.C. Kavanagh, M. Pintilie, A. Sun, W. Chapman, W. Levin, L. Manchul, T.J. Keane, R.P. Hill, Radiother. Oncol. 48 (1998) 149–156;
(b) H.A. Al-Hallaq, J.N. River, M. Zamora, H. Oikawa, G.S. Karczmar, Int. J. Radiat. Oncol. Biol. Phys. 41 (1998) 151–159;
(c) A.Y. Karen, B.P. Siham, M.L. Rachelle, Q.B. Darrell, C.F. Matthew, S.B. James, E.H. Gerald, J.D. Chapman, Int. J. Radiat. Oncol. Biol. Phys. 33 (1995) 111–118;
(d) A.S.E. Ljungkvist, J. Bussink, P.F.J.W. Rijken, J.A. Raleigh, J. Denekamp, A.J. van der Kogel, Int. J. Radiat. Oncol. Biol. Phys. 48 (2000) 1529–1538.
[7] (a) J.A. Raleigh, A.J. Franko, C.J. Koch, J.L. Born, Br. J. Cancer 51 (1985) 229–235;
(b) J.D. Chapman, A.J. Franko, J.A. Sharplin, Br. J. Cancer 45 (1982) 694–699;
(c) B.M. Garrecht, J.D. Chapman, Br. J. Radiol. 56 (1983) 745–753;
(d) J.S. Rasey, K.A. Krohn, Z. Grunbaum, P.J. Conroy, K. Bauer, R.M. Sutherland, Radiat. Res. 102 (1985) 76–85;
(e) Z. Li, T. Chu, X. Liu, X. Wang, Nucl. Med. Biol. 32 (2005) 225–231;
(f) M. Dai, W. Zhu, Y. Xu, X. Qian, J. Fluoresc 18 (2008) 591–597;
(g) W. Zhu, M. Dai, Y. Xu, X. Qian, Bioorg. Med. Chem. 16 (2008) 3255–3260.
[8] A.C. Begg, E.L. Engelhardt, R.J. Hodgkiss, N.J. McNally, N.H.A. Terry, P. Wardman, Br. J. Radiol. 56 (1983) 970–973.
[9] P.L. Olive, R.E. Durand, Cancer Res. 43 (1983) 3276–3280.
[10] P.L. Olive, Int. J. Radiat. Oncol. Biol. Phys. 10 (1984) 1357–1360.
[11] M.R.L. Stratford, E.D. Clarke, R.J. Hodgkiss, R.W. Middleton, P. Wardman, Int. J. Radiat. Oncol. Biol. Phys. 10 (1984) 1353–1356.
[12] P. Wardman, E.D. Clarke, R.J. Hodgkiss, R.W. Middleton, J. Parrick, M.R.L. Stratford, Int. J. Radiat. Oncol. Biol. Phys. 10 (1984) 1347–1351.
[13] A.C. Begg, R.J. Hodgkiss, N.J. McNally, R.W. Middleton, M.R.L. Stratford, N.H.A. Terry, Br. J. Radiol. 58 (1985) 645–654.
[14] (a) P.L. Olive, D.J. Cha-din, Int. J. Radiat. Oncol. Biol. Phys. 12 (1986) 1247–1250.
[15] R.J. Hodgkiss, R.W. Middleton, M.R.L. Stratford, R.D. Buono, Biochem. Pharmcol. 36 (1987) 1483–1487.
[16] M.F. Braña, M. Cacho, A. Gradillas, B. de Pascual-Teresa, A. Ramos, Curr. Pharm. Des. 7 (2001) 1745–1780.
[17] (a) M. Hockel, K. Schlenger, C. Knoop, P. Vaupel, Cancer Res. 51 (1991) 6098–6102;
(b) W. Mueller-Klieser, P. Vaupel, R. Manz, R. Schmidseder, Int. J. Radiat. Oncol. Biol. Phys. 7 (1981) 1397–1404;
(c) P. Vaupel, K. Schlenger, C. Knoop, M. Hockel, Cancer Res. 51 (1991) 3316–3322.
[18] (a) W.A. Denny, W.R. Wilson, M.P. Hay, Br. J. Cancer 74 (1996) 32–38;
(b) W.A. Denny, W.R. Wilson, J. Pharm, Pharmacology 50 (1988) 387–394.
[19] J.M. Brown, W.R. Wilson, Nat. Rev. Cancer 4 (2004) 437–447.
[20] (a) L.H. Patterson, Cancer Metastasis. Rev. 12 (1993) 119–134;
(b) A.K. Sinhababu, D.R. Thakker, Adv. Drug. Deliv. Rev. 19 (1996) 241–273.
[21] (a) K. Král, P. Zdenk, Microelectron. Engineer 69 (2003) 256–260;
(b) E. Gaiauskas, K.H. Feller, J. Lumin 102-103 (2003) 13–20;
(c) K.H. Feller, E. Gaiauskas, J. Lumin 83 (1999) 265–270;
(d) K.R. Naqvi, T.B. Melo, B.B. Raju, T. Javorfi, G. Garab, Spectrochim. Acta Part A: Mol. Biomol. Spectrosc. 53 (1997) 1925–1936.
[22] W. Chen, Y. Qin, Z. Cai, C. Chan, G. Luo, Z. Jiang, Bioorg. Med. Chem. 13 (2005) 1859–1866.
[23] (a) R.J. Hodgkiss, A.C. Begg, R.W. Middleton, J. Parrick, M.R.L. Stratford, P. Wardman, G.D. Wilson, Biochem. Pharmacol. 41 (1991) 533–541;
(b) R.J. Hodgkiss, R.W. Middleton, J. Parrick, H.K. Rami, P. Wardman, G.D. Wilson, J. Med, Chem 35 (1992) 1920–1926;
(c) R.J. Hodgkiss, J. Parrick, P. Manuchehr, M.R.L. Stratford, J. Med. Chem. 37 (1994) 4352–4356.
[24] W.R. Wilson, P. van Zijl, W.A. Denny, Int. J. Radiat. Oncol. Biol. Phys. 22 (1992) 693–696.
[25] D.C. Heimbrook, A.C. Sartorelli, Mol. Pharmacol. 26 (1986) 168–172.
[26] Y. Xu, B. Qu, X. Qian, Y. Li, Bioorg. Med. Chem. Lett. 15 (2005) 1139–1142.
[27] X. Qian, Y. Li, Y. Xu, Y. Liu, B. Qu, Bioorg. Med. Chem. Lett. 14 (2004) 2665–2668.
[28] Y.S. Bcheshtiha, M.M. Heravi, F.A. Salehi, Indian. J. Chem. 37B (1998) 1177–1179.